

**Figure S1. BCAA Catabolism Is Suppressed in Hepatocellular Carcinomas and Predicts Patient Survival, Related to Figure 1**

(A) Patient characteristics of the cohort recruited at Singapore General Hospital (SGH), including total number, average age (range), and number (percent) of males, females, Hepatitis B\*, Hepatitis C\*, and indicated racial backgrounds. Additional information on tumor characteristics of the cohort can be found in Figure 1H.

(B) Summary of differential expression analysis from paired HCCs and nontumor liver tissues of the SGH cohort.

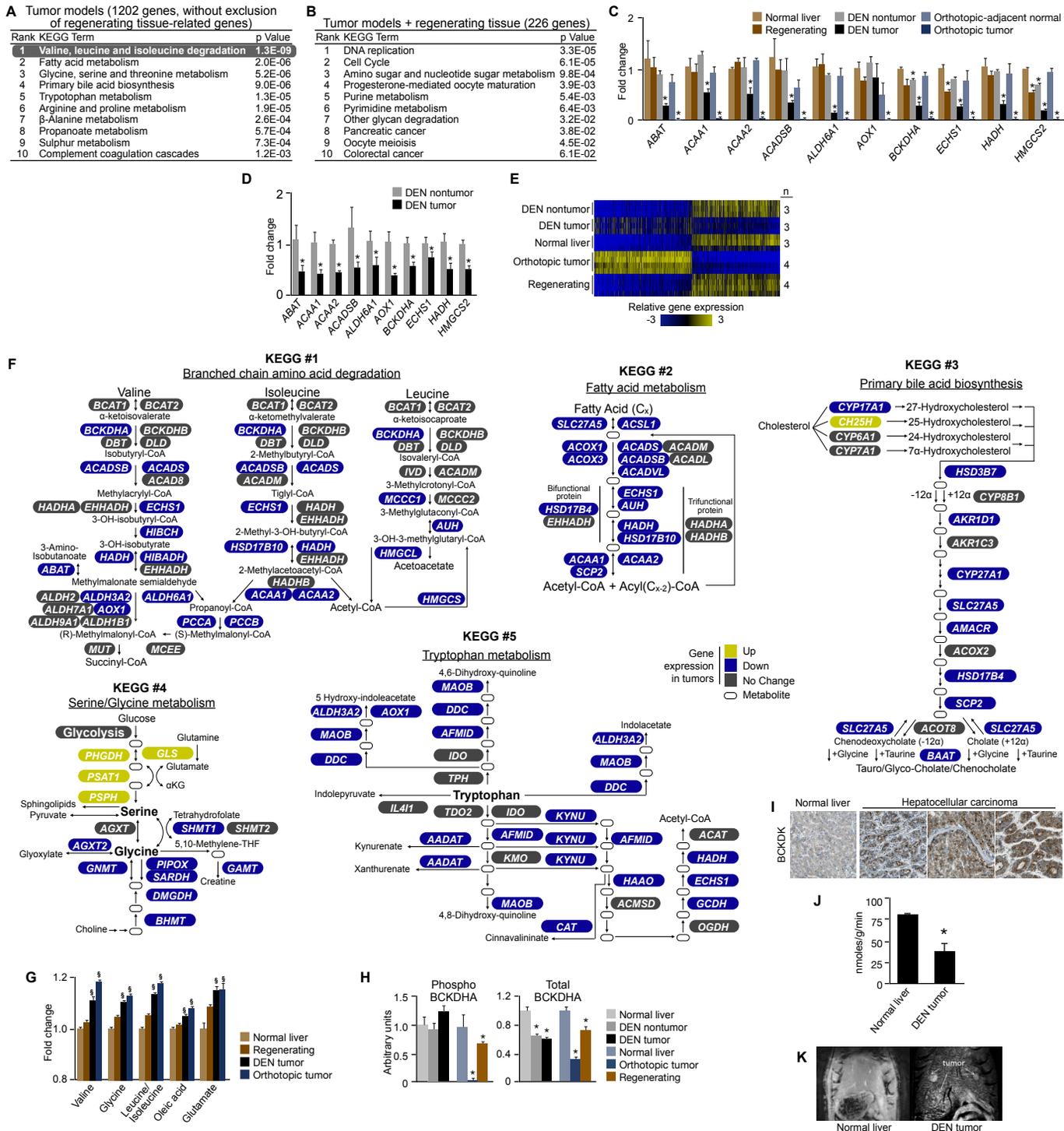
(C-E) Immunoblots for BCAT1 and BCAT2 in (C) HCCs and nontumor liver tissues from patients of the SGH cohort, (D) liver-derived cancer cell lines, and (E) normal, tumor, and regenerating liver tissues of animal models.

(F) Representative immunohistochemical micrographs from nontumor liver tissue and HCC biopsies, as profiled by The Human Protein Atlas.

(G) Summary of metabolites that were not significantly different in paired HCCs and nontumor liver tissues from patients of the SGH cohort. Data are shown as mean  $\pm$  s.e.m.

(H) Summary of BCAA catabolic enzyme transcript levels in HCCs from the SGH and TCGA cohorts, sorted by race/ethnicity, tumor etiology, and extratumoral liver inflammation, as well as the association of these characteristics with tumor aggressiveness.

(I) Quantification of cox proportional hazard ratios (95% confidence intervals), significance (log-rank P-value), robustness, and difference in days of estimated survival for patients of the TCGA-LIHC cohort with low expression of indicated BCAA catabolic enzymes.



**Figure S2. Loss of BCAA Catabolism Occurs in Liver Cancers but Not Regenerating Liver Tissues, Related to Figure 2**

(A) KEGG pathway analysis of all 1202 genes significantly different in DEN and orthotopic (Morris Hepatoma) tumor models (without exclusion of genes significantly different in regenerating tissues).

(B) KEGG pathway analysis of the 226 genes shared by regenerating tissues, and DEN and orthotopic tumors.

(C) RT-PCR analysis of BCAA catabolic enzymes from rat tumor and regenerating tissues, normalized to normal liver tissues.

(D) RT-PCR analysis of BCAA catabolic enzymes from mouse tumor tissues, normalized to nontumor liver tissue.

(E) Expression summary of all 976 genes identified in the transcriptomic analysis. DEN tumor tissues are compared to DEN nontumor tissues (mouse), and Morris Hepatoma and regenerating liver tissues are compared to normal liver tissues (rat).

(F) Summary of expression changes in the top five KEGG pathways.

(G) Non-targeted metabolomics analysis of rat DEN-induced tumors, Morris hepatoma tumors, and regenerating tissues, normalized to normal liver tissues.

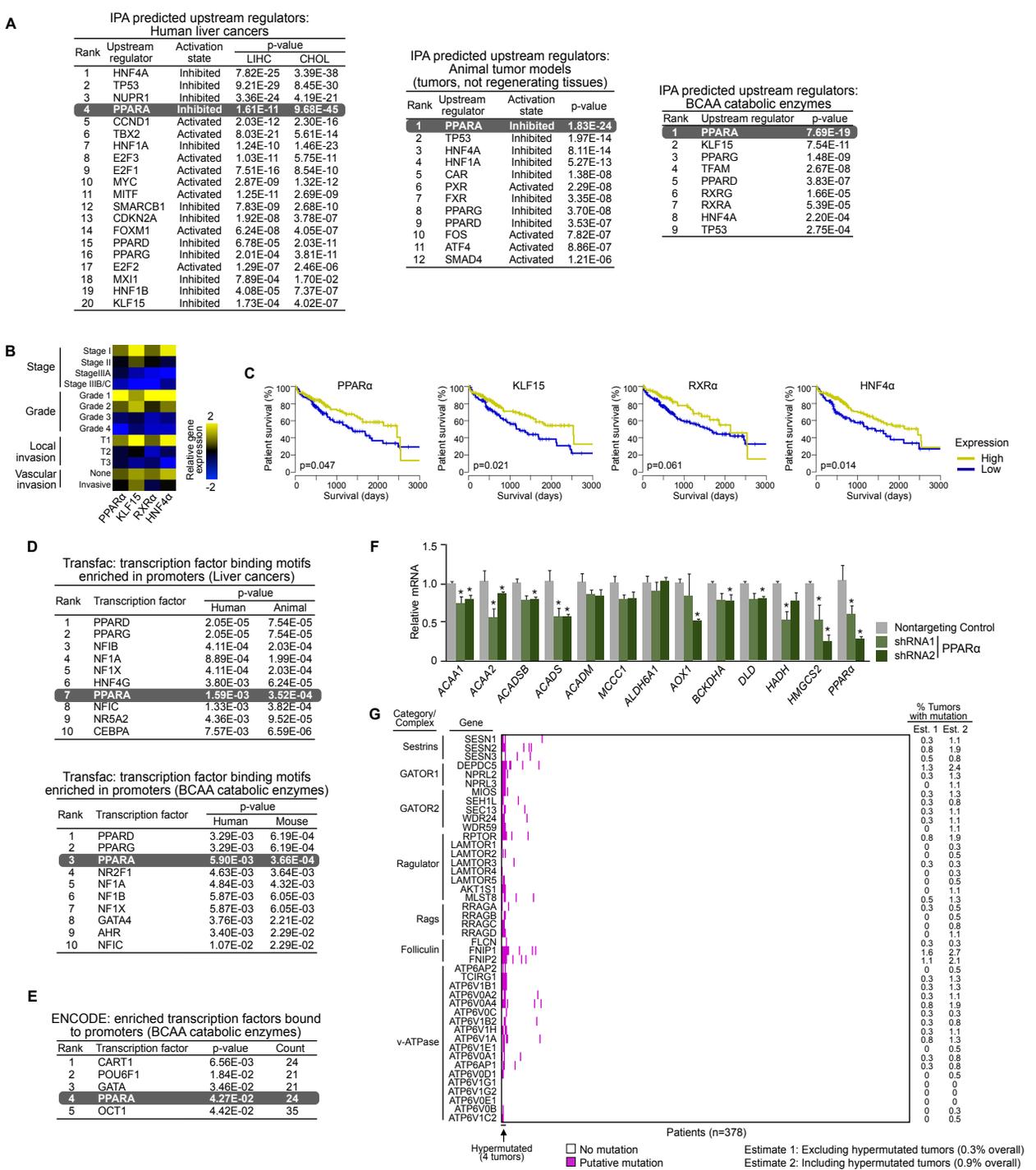
(H) Quantification of immunoblots presented in Figure 2F, normalized to respective normal liver tissue controls.

(I) Representative BCKDK immunohistochemical micrographs from nontumor liver tissue and HCC biopsies, as profiled by The Human Protein Atlas.

(J) Quantification of ex vivo BCKDH complex activity in normal liver tissues and DEN-induced tumors in rats.

(K) Representative coronal FLASH MRI images of DEN-induced liver tumors and normal liver from rats used in the MRS analyses.

\* $P < 0.05$ , § $P < 0.01$ , compared to respective controls. Data are shown as mean  $\pm$  s.e.m.



**Figure S3. Reduced BCAA Catabolic Enzyme Expression Is Associated with Copy Number Variations and Transcription Factor Alterations, Related to Figure 3**

(A) Ingenuity Pathway Analysis identifying predicted upstream regulators of all significant, differentially expressed genes of TCGA human liver cancers, animal models (different in tumors but not regenerating tissues), and specifically the BCAA catabolic enzymes.

(B) Summary of transcription factor expression in HCCs from the TCGA-LIHC cohort, sorted by stage, grade, vascular invasion, and local invasion.

(C) Kaplan-Meier survival estimate curves for TCGA-LIHC patients ranked by expression of indicated transcription factors. P-values for log-rank test shown.

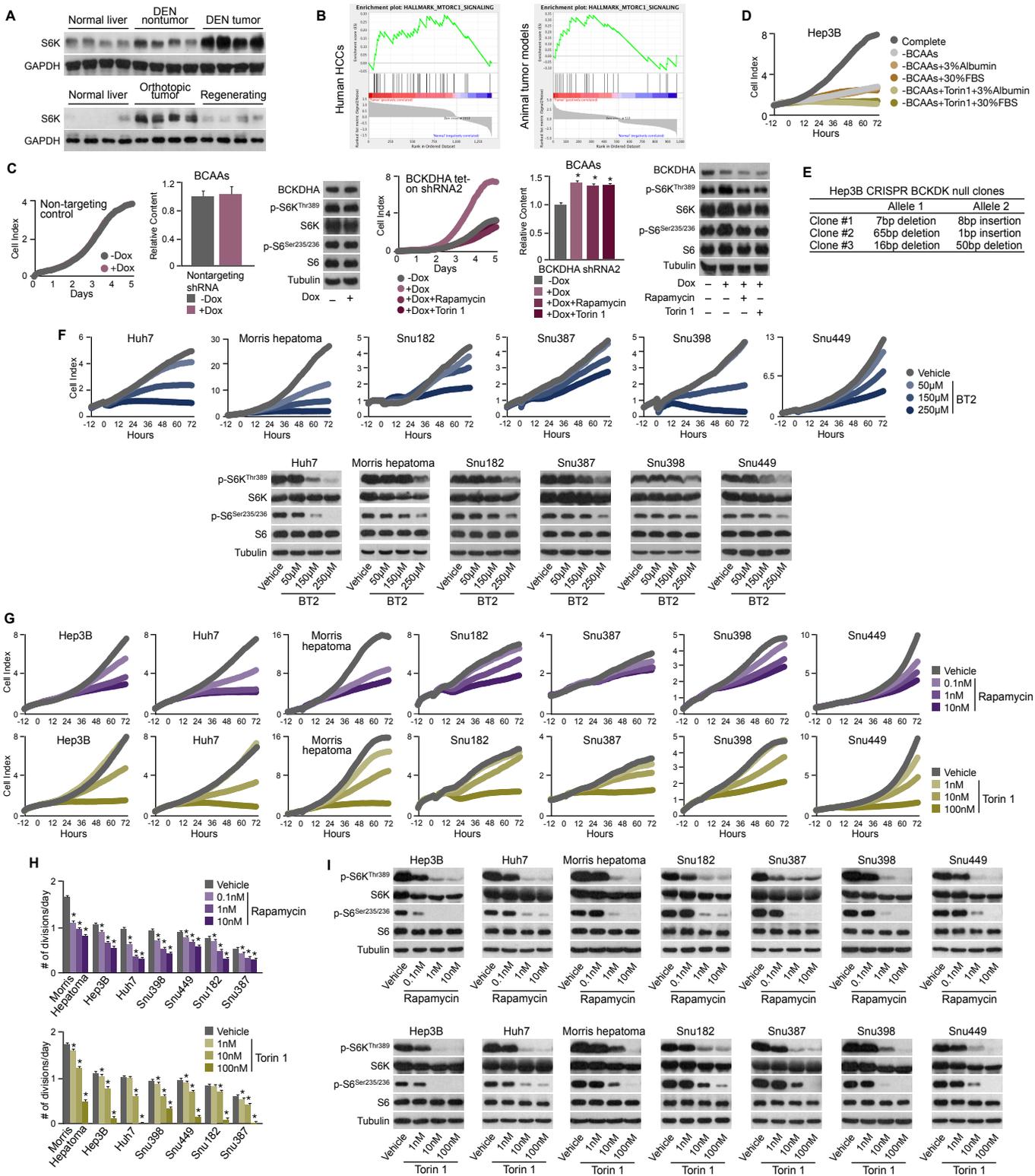
(D) Transfac analysis identifying enriched transcription factor sequence motifs in the promoters of all significant, differentially expressed genes of human liver cancers, animal models (different in tumors but not regenerating tissues), and specifically the BCAA catabolic enzymes.

(E) Summary of ENCODE ChIP-seq data identifying enriched transcription factors bound to the promoters of BCAA catabolic enzymes.

(F) RT-PCR analysis of BCAA catabolic enzymes from HepG2 cells expressing shRNAs to PPAR $\alpha$  or nontargeting control.

(G) Summary of all non-silent mutations of the TCGA-LIHC in proteins related to the nutrient-sensing arm of mTORC1.

\*P<0.05, compared to respective controls. Data are shown as mean  $\pm$  s.e.m.



**Figure S4. BCAA Catabolism Regulates mTORC1 Activity and *in vitro* Cell Proliferation, Related to Figure 4**

(A) Immunoblots of the mTORC1 downstream effector S6K in animal liver tumor and regenerating models.

(B) "mTORC1 signaling" enrichment plots from gene set enrichment analysis (GSEA) of the 1405 human HCC and 976 animal tumor model (nor regenerating) data sets.

(C) Real-time proliferation curves, immunoblots detailing knockdown efficiency (BCKDHA) and mTORC1 pathway activity (p-S6K<sup>Thr389</sup> to total S6K, and p-S6<sup>Ser235/236</sup> to total S6 ratios), and intracellular BCAA content of AML12 cells expressing a tet-inducible non-targeting control or BCKDHA shRNAs, in the absence or presence of doxycycline and/or the mTOR inhibitors rapamycin (0.05nM) or Torin 1 (0.5nM).

(D) Real-time proliferation curves of Hep3B cells grown in complete or BCAA-free media, with or without 3% albumin, 30% FBS, and/or 100nM Torin 1. Similar results were obtained when using Leucine-free media and/or Rapamycin.

(E) Summary of frame-shift mutations caused by CRISPR-Cas9-mediated insertions and/or deletions in the BCKDK gene of Hep3B clones.

(F) Real-time proliferation curves of the liver cancer cell lines treated with the BCKDK inhibitor BT2, and immunoblots after 2 hours of treatment.

(G-I). Characterization of liver cancer cell lines treated with the mTOR inhibitors Rapamycin or Torin 1. (G) Representative real-time proliferation curves. (H) Calculation of proliferation rates (number of divisions per day). (I) Immunoblots after 2 hours of treatment.

\*P<0.05, compared to respective controls. Data are shown as mean ± s.e.m.



**Figure S5. Characterization of Mice on BCAA-Supplemented or -Restricted Diets, Related to Figures 5 and 6**

(A) Summary of RT-PCR statistical analyses presented in Figure 5H, comparing tissues of LFD+BCAA, HFD, and HFD+BCAA groups to corresponding tissues of the LFD group.

(B) Quantification of nontumor liver tissue amino acid content of DEN-injected mice fed indicated diets, 5 months post injection, normalized to the LFD group. \*P<0.05 vs. LFD. §P<0.05 vs. HFD.

(C) Quantification of tumor and nontumor liver tissue amino acid content of DEN-injected mice fed indicated diets, 8 months post injection, normalized to respective LFD groups. \*P<0.05 vs. LFD.

(D) Quantification of tumor and nontumor liver tissue acylcarnitine content from DEN-injected mice fed indicated diets, 8 months post injection, normalized to nontumor tissue of the LFD group. \*P<0.05 vs. nontumor LFD, §P<0.05 vs. tumor LFD.

(E) RT-PCR analysis of cytokines and F4/80 (encoded by *ADGRE1*) in normal liver tissue (from uninjected mice), and nontumor and tumor liver tissues (from DEN-injected mice). Results normalized to normal liver tissue of LFD-fed mice.

(F) Representative histological analyses of livers from DEN-injected mice fed indicated diets, 5 months post injection.

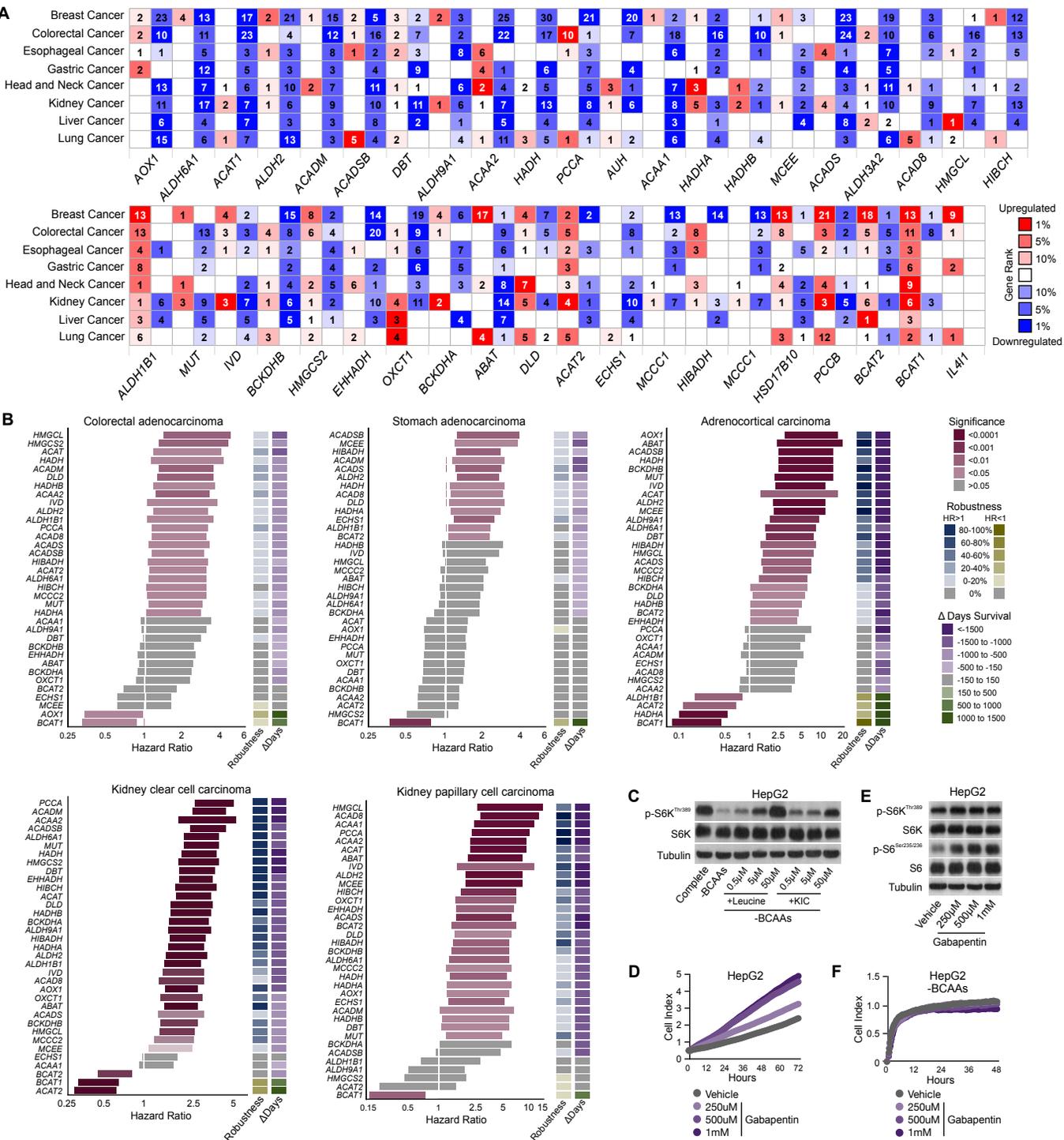
(G) Quantification of immunohistochemical staining related to Figures 5K and S5G as percent area (picosirius red) or percent positive cells (remaining), n ≥ at least two random fields per sample, and at least 3 samples per group.

(H) Characterization of DEN-injected mice fed standard chow diets, or chow diets with 0.02% BT2 added, including quantification of the number of tumors (≥3mm) and size of the largest tumor per mouse.

(I) Summary of RT-PCR statistical analyses presented in Figure 6I, comparing tissues of LFD-lowBCAA and LFD+BCAA groups to corresponding tissues of the LFD group.

(J) Quantification of immunohistochemical staining related to Figure 6G as percent area (picosirius red) or percent positive cells (remaining), n ≥ at least two random fields per sample, and at least 3 samples per group.

Data are shown as mean ± s.e.m.



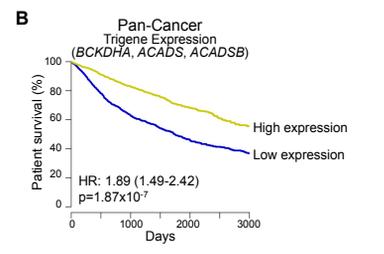
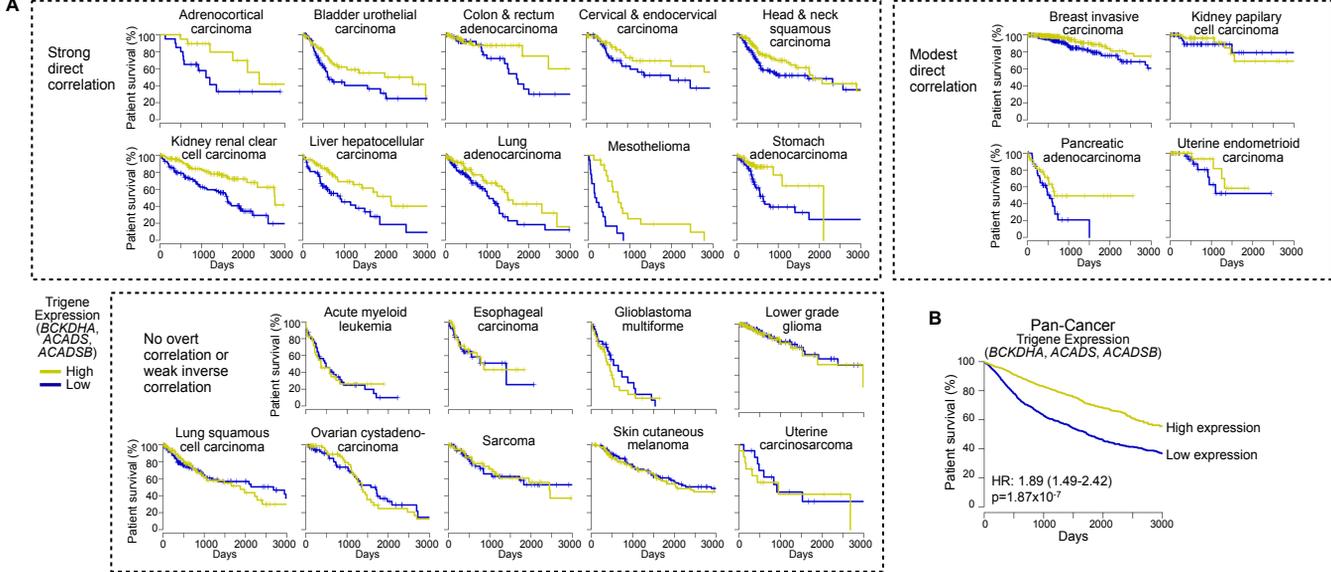
**Figure S6. The Impact of Tissue BCAA Catabolism on Cancer Development and Progression, Related to Figure 7**

(A) Oncomine analysis of mRNA expression datasets profiling various cancers compared to nontumor controls. Colors reflect the degree of over- or under-expression, and numbers reflect the quantity of studies included in the summation.

(B) Quantification of cox proportional hazard ratios (95% confidence intervals), significance (log-rank P-value), robustness, and difference in days of estimated survival for patients of the TCGA COADREAD, STAD, ACC, KIRC, and KICP cohorts with low expression of indicated BCAA catabolic enzymes.

(C) Immunoblots for mTOR pathway activity in HepG2 cells cultured in complete media, or cultured in BCAA-free media for 4 hours, then stimulated with indicated concentrations of leucine or  $\alpha$ -ketoisocaproate (KIC) for 10 minutes.

(D-F) BCAT1 inhibition in HepG2 cells. (D) Real-time proliferation curves of HepG2 cells grown in complete media treated with the BCAT1 inhibitor gabapentin. (E) Immunoblots of HepG2 cells 4 hours after gabapentin treatment. (F) Real-time proliferation curves of HepG2 cells grown in BCAA-free media treated with gabapentin.



**C**

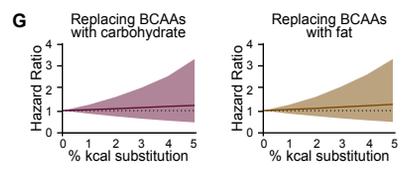
	Entire Sample	Low BCAA (<1.73% kcal)	Medium BCAA (1.73-3.89% kcal)	High BCAA (>3.89% kcal)
N	3156	277	2548	331
Age (years)	57.3 (0.15)	56.9 (0.41)	57.3 (0.15)	57.0 (0.38)
Female (%)	52.3	49.5	52.4	54.4
High School Graduate (%)	53.5	49.5	54.6	43.8
Non-Hispanic White (%)	45.6	43.0	46.3	42.6
Non-Hispanic Black (%)	25.7	33.9	24.8	25.4
Mexican American (%)	24.3	18.4	24.5	27.2
Current Smoker (%)	26.0	27.8	26.3	23.0
Waist Circumference (cm)	97.4 (0.38)	97.3 (1.14)	97.3 (0.42)	98.0 (0.97)
Total Physical Activity (METs)	101.2 (4.27)	84.0 (8.15)	100.4 (4.36)	120.4 (14.4)
BCAA (%)	2.78 (0.02)	1.46 (0.02)	2.69 (0.01)	4.54 (0.06)
Non BCAA (%)	13.2 (0.11)	7.53 (0.08)	12.8 (0.07)	21.0 (0.29)
Fat (%)	33.7 (0.28)	31.2 (1.02)	34.2 (0.30)	31.3 (0.75)
Carbohydrate (%)	49.6 (0.33)	56.1 (1.26)	49.8 (0.32)	42.8 (0.86)
Cancer Mortality (%)	11.9	10.5	11.9	13.0
Person Years	54,781	4,934	44,332	5,515

**E**

Tertile	BCAA Intake (% kcal)	Adjusted HR <sup>1</sup>	Adjusted HR <sup>2</sup>
1	Low (<1.73%)	1.0	1.0
2	Medium (1.73-3.89%)	0.78 (0.54-1.12)	0.94 (0.62-1.45)
3	High (>3.89%)	0.55 (0.32-0.94)	0.92 (0.40-2.12)

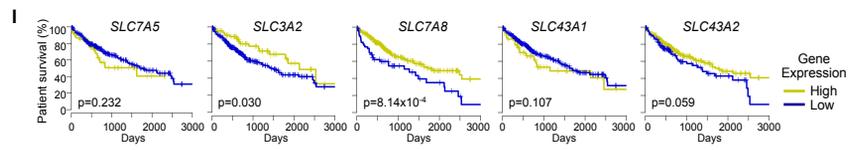
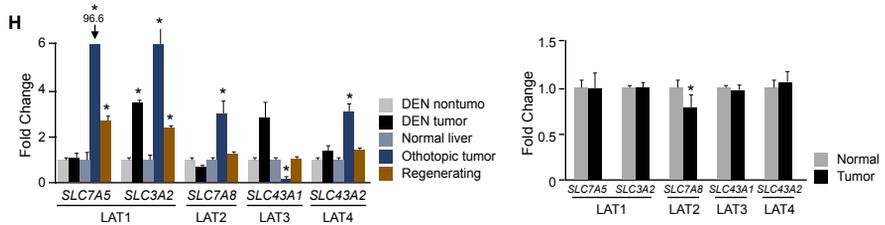
**F**

Protein Source	Adjusted HR <sup>1</sup>	Adjusted HR <sup>2</sup>
BCAA	0.82 (0.71-0.95)	1.00 (0.80-1.25)
Non BCAA	0.95 (0.92-0.98)	0.99 (0.95-1.04)



**D**

Protein Source	Adjusted HR <sup>1</sup>	Adjusted HR <sup>2</sup>
BCAA	1.29 (1.08-1.55)	1.27 (1.03-1.57)
Non BCAA	1.07 (1.03-1.11)	1.06 (1.01-1.12)



**Figure S7. The Impact of BCAA Tissue Catabolism and Dietary Intake on Overall Cancer Mortality, Related to Figure 7**

(A) Kaplan-Meier survival estimate curves of all cancers profiled by TCGA with at least 15 verified deaths. Within each cancer, individuals were ranked by a combined expression index of *ACADS*, *ACADSB*, and *BCKDHA*, and top and bottom quartiles were compared.

(B) Kaplan-Meier survival estimate curve of the normalized pan-cancer dataset by TCGA. Individuals were ranked by a combined expression index of *ACADS*, *ACADSB*, and *BCKDHA*, and top and bottom quartiles were compared. Cox proportional hazard ratio (HR) for low expression group, and p-value for log-rank test shown.

(C) Population characteristics of NHANES III participants, aged 50-66 years old. Data presented as mean (with standard error).

(D) Change in cancer mortality risk associated with increasing BCAA or non BCAA protein content by 1% kcal in the 50-66 year old cohort.

(E-G) Analysis of cancer mortality risk of individuals >66 years-old. (E) Hazard ratios (with 95% confidence interval) based on BCAA intake, <sup>1</sup>adjusted for age, sex, race, total kcal, usual dietary intake, diet change, physical activity, intentional weight loss, waist circumference, smoking, education, and prior diagnosis of cancer, diabetes and cardiovascular disease, <sup>2</sup>additionally adjusted for % kcal from other macronutrients. (F) Change in cancer mortality risk associated with increasing BCAA or non-BCAA protein content by 1% kcal. (G) Substitution analysis comparing change in risk when replacing BCAAs with carbohydrate or fat, with the same adjustments as HR<sup>2</sup> except total kcal and % kcal from non-BCAA protein. Data are presented as hazard ratio (solid line) with 95% confidence interval (shaded area).

(H) Summary of LAT transporter expression in the animal tumor and regenerating models (mean ± s.e.m.), and human HCC (mean ± s.d.; TCGA-LIHC cohort).

(I) Kaplan-Meier survival estimate curves for patients ranked by tumor LAT transporter expression. P-values for log-rank test shown.

\*P<0.05, compared to respective controls.

Study	Diet	Catalog Number	Fat		Carbohydrate		Protein	
			% kcal	Composition	% kcal	Composition	% kcal	Composition
#1	LFD	D07010502	10%	55% soybean oil,	71%	45% corn starch, 5%	19%	53% casein; 47% purified amino acids
	LFD+BCAA	D07010503	10%	45% lard	67%	maltodextrin, 50% sucrose	23%	41% casein; 58% purified amino acids
	HFD	D06011802	45%	12% soybean oil,	35%	21% corn starch, 29%	19%	53% casein; 47% purified amino acids
	HFD+BCAA	D06050807	43%	88% lard	34%	maltodextrin, 50% sucrose	23%	41% casein; 58% purified amino acids
#2	LFD+BCAA	A14100108	10%	55% soybean oil,	69%	61% corn starch, 14%	21%	100% purified amino acids
	LFD+BCAA+0.02%BT2	A15012814	10%	45% lard	69%	maltodextrin, 25% sucrose	21%	
	HFD+BCAA	A14100104	44%	12% soybean oil,	35%	21% corn starch, 29%	21%	
	HFD+BCAA+0.02%BT2	A15012820	44%	88% lard	35%	maltodextrin, 50% sucrose	21%	
#3	LFD-lowBCAA	A14100106	10%	55% soybean oil,	75%	64% corn starch, 13%	15%	100% purified amino acids
	LFD-lowAA*	A14100105	10%		75%	maltodextrin, 23% sucrose	15%	
	LFD+AA*	A14100107	10%		69%	61% corn starch, 14%	21%	
	LFD+BCAA	A14100108	10%		69%	maltodextrin, 25% sucrose	21%	

**Table S1. Rodent Diet Composition, Related to STAR Methods**

Summary of % of kcal, and source of the fat, carbohydrate, and protein used in the rodent studies. Diets with supplemented BCAAs (+BCAA) included an additional 150% of purified leucine, isoleucine, and valine over baseline levels. Diets with restricted BCAAs (-lowBCAA) lowered leucine, isoleucine, and valine to 50% of baseline levels. \*LFD-lowAA and LFD+AA diets were used to control for any possible effects of total protein content in LFD-lowBCAA and LFD+BCAA diets, respectively. Baseline BCAA levels were maintained, while all other amino acids were adjusted proportionally.

Human		Mouse		Rat	
Target	Sequence	Target	Sequence	Target	Sequence
ABAT	5'-TGCTCCCTACCCGATCTTCA-3' 5'-GTCCTCCCGCTTGTGATGT-3'	ABAT	5'-GAGGCCGTGCACATTTTCTG-3' 5'-CCAGAGCCGGATGGTTGTA-3'	ABAT	5'-GAGGCCGTGCACATTTTCTG-3' 5'-CGCGTTTTGAGGCTGTTGAA-3'
ACAA1	5'-AATGAGGCCTTGAAGCCA-3' 5'-AGCGTGATGACCTGTCTGG-3'	ACAA1	5'-ACAGTGTTCATCGGGACTGC-3' 5'-AGATAATCCAGGGTCCCA-3'	ACAA1	5'-TTACGACATTGGCATGGCT-3' 5'-CAGCCACATTCTCCGAGGT-3'
ACAA2	5'-TCTGCTGGCAAAGTCTACC-3' 5'-CAAACCAACATGCCTTGCCA-3'	ACAA2	5'-ACATAACTTCACGCCCTGG-3' 5'-GAGGGGCAAAGCTTCTGTC-3'	ACAA2	5'-GGCAAAGTTCACCGGAAAC-3' 5'-ACTGAAACAGAGCCACAG-3'
ACADM	5'-GATGTGGATAACCAACGGAGGA-3' 5'-CCTGGGTATCTGCTTCCAC-3'	ACADS	5'-TTGCCGAGAAGGAGTTGGTC-3' 5'-AGGAATCCAAGCCTGCACC-3'	ACADS	5'-AGCCTTTCACCAAGGATGCG-3' 5'-CATCTCGGTAGTAGCGTCG-3'
ACADS	5'-CCTCGATTACTGGCCTACG-3' 5'-TCTGCTCCTGGAGCCAAAC-3'	ACADSB	5'-GGACTGGCCCAAGGATGTTT-3' 5'-CGAGCCTAGCAGCGTTGTAT-3'	ACADSB	5'-GAGACTGGCCCAAGGATGTTT-3' 5'-ATAAATGGCCTCCCGGCTC-3'
ACADSB	5'-TTGGGGCTCAGAGCTTCTTC-3' 5'-AGCCATGTCCAATTTGCCCA-3'	ALDH6A1	5'-CGTGGGCAGACACATCTAT-3' 5'-CTCCAGCATGAGGGATGTC-3'	ALDH6A1	5'-AGTACCTGGAGCAACCATGC-3' 5'-CGAAGATGTACTCTCCGCCC-3'
ALDH6A1	5'-CAGGTGGGAGTGAATGTCCC-3' 5'-AATTGGATGCCTGTTGCC-3'	AOX1	5'-TACGTGAATGGCCAGAAGGT-3' 5'-GGATGATGCCTGATCGCCTT-3'	AOX1	5'-ACCGTACTGAGGAAGACCT-3' 5'-TGCCCTCTACTGTGGTACT-3'
AOX1	5'-AATAGTCCACTGACCCCGGA-3' 5'-CTGGGAAGAGGCACTCTGTT-3'	BCKDHA	5'-GACCTGGTGTGGCCAGTA-3' 5'-GCCGTAGTGAACAGGCATCT-3'	BCKDHA	5'-CAACGATGTGTTGCGGTGT-3' 5'-TTGACCTCATCCACCGAACG-3'
BCKDHA	5'-TGGATGACAAGCCCAAGTTC-3' 5'-GCGGTAGATGGGATTCCAG-3'	ECHS1	5'-CTCTTGGTGGGGTGTGAA-3' 5'-TTGTAGCGGATTGCGCACT-3'	ECHS1	5'-AGGCCATCCAATGTGCAGAA-3' 5'-TCCACAAGGCAGACATCCC-3'
DLD	5'-TGGCCGACGACCCCTTACTA-3' 5'-GCTTTGTGAGCCAGCATTTGG-3'	HADH	5'-GACAAGACCGATTGCTGGC-3' 5'-CACCAGAGTCGGTTCACGA-3'	HADH	5'-GACAAGACCGATTGCTGGC-3' 5'-CACCAGAGTCGGTTCACGA-3'
HADH	5'-ACCAGACAAGACCGATTGCG-3' 5'-TCTTCTGGCTGGTACTTGT-3'	HIBCH	5'-AGCGCTCATAACGCTCAAC-3' 5'-TCTCCGGCTCCCTTTATGA-3'	HIBCH	5'-TGGAACAGATTAAGTTCTCATTGAC-3' 5'-TATGGTGTGCTGACCCCTTG-3'
HMGCS2	5'-TCCATCCAGTCTACTTGC-3' 5'-ATCGTCAAGGGTGAAGGGTC-3'	HMGCS2	5'-GCCCAAACGTCTAGACTCCC-3' 5'-CTCCATTAGACGGGACCCG-3'	HMGCS2	5'-GCCCAAACGTCTAGACTCCC-3' 5'-CGGGCATATTTCTGCGGTG-3'
MCCC1	5'-ACAATGTAGCCATAGCTGTAACG-3' 5'-GCAGTCTCCCTCGCTGAAA-3'	MCCC1	5'-TGGGGTAGCCCGTAATCCA-3' 5'-GAGCTCCTTCTGCACCTCACT-3'	MCCC1	5'-AAGGCATGTGGAAGTCCAGG-3' 5'-CAGGATCAATACCAGGCGCT-3'
PPARα	5'-ATCCCAGGCTTCGCAACTT-3' 5'-CATGGCGAATATGGCCTCAT-3'	β-ACTIN	5'-CAAGGTCATCCATGACAACCTTTG-3' 5'-GGCCATCCACAGTCTTCTGG-3'	β-ACTIN	5'-CAAGGTCATCCATGACAACCTTTG-3' 5'-GGCCATCCACAGTCTTCTGA-3'
β-ACTIN	5'-CAAGGTCATCCATGACAACCTTTG-3' 5'-GGCCATCCACAGTCTTCTGG-3'	GAPDH	5'-CAAGGTCATCCATGACAACCTTTG-3' 5'-GGCCATCCACAGTCTTCTGG-3'	GAPDH	5'-CAAGGTCATCCATGACAACCTTTG-3' 5'-GGCCATCCACAGTCTTCTGA-3'
GAPDH	5'-GCTCTGCTGCTCCTGTTC-3' 5'-ATGGTGTCTGACGATGTGG-3'	ADGRE1	5'-CTTGTCTATGGCTTCCAGT-3' 5'-GCAAGGAGGACAGAGTTTATCGT-3'		
		IFNγ	5'-AGCAACAGCAAGGCGAAAAG-3' 5'-CGCTTCTGAGGCTGGATTC-3'		
		IL-1β	5'-CAAGCAACGACAAAATACCTGTG-3' 5'-AGACAACCGTTTTTCCATCTTCT-3'		
		IL-4	5'-GTGCCAAACGTCCTCACAGC-3' 5'-CTGACGCTCCATGAGAACACTAGA-3'		
		IL-6	5'-CCGAGAGGAGACTTCACAGAG-3' 5'-CTGACAGTGCATCATCGTGT-3'		
		TNFα	5'-TGCCCAAGCCCTCACACTCAG-3' 5'-ACCCATCGGCTGGCACCCT-3'		

**Table S2. Real-time PCR Primer Sequences, Related to STAR Methods**

Primer sequences used for human, mouse, and rat samples.